## Remarks

Claims 2, 5, 7-9, and 18-25 are canceled herein. Claims 1, 3-4, 6, 10-17 and 26 are amended herein. New claim 27 is added herein.

Support for the amendment of claim 1 can be found throughout the specification, for example at page 5, lines 10-30, page 26, lines 14-29 and page 31, line 22 to page 32, line 23. Support for the amendments of claims 3-4, 6, 12, 16-17 and 26 can be found throughout the specification, for example at page 5, lines 10-30. Claims 10 and 13-14 are amended to correct form. Claim 11 is amended to correct dependency. Support for the amendment of claim 15 can be found throughout the specification, for example on page 23, line 3 to page 25, line 21 and pages 31-32.

No new matter is added. Reconsideration of the subject application is respectfully requested.

### *Restriction Requirement*

Applicants thank the Examiner for noting that claims 1-6, 9-18 and 26 were included in Group I. Claims 7-8 and 19-25 are canceled herein in response to the restriction requirement.

### *Objection to Claim 5*

Claim 5 is objected to for including non-elected sequence identifiers. Claim 5 is canceled herein, rendering the objection moot.

*Rejections Under 35 U.S.C. § 101*

Claims 1-6 and 9-18 were rejected as allegedly lacking a utility that was specific, substantial and credible. Claims 2, 5, and 7-9 are canceled herein, rending this rejection moot as applied to these claims. Applicants respectfully disagree with this rejection as applied to the amended claims or as may be applied to the newly added claims.

The Office action asserts that the specification does not assert a utility for all transgenic animals including a transgene encoding NOX1. Applicants respectfully disagree with the rejection as applied to the claims as amended.

The claims are amended to be directed to transgenic mice harboring a transgene that includes a nucleic acid sequence set forth as SEQ ID NO: 1 or a degenerate variant thereof operatively linked to a promoter. These mice exhibit an increased overgrowth of colonic epithelial cells upon exposure to pathogenic bacteria. As disclosed in Example 10, Example 11, and Fig. 4 (see the specification at pages 31-32), these mice heterozygous for a transgene encoding NOX 1 have an increased hyperplastic response in the colon in response to a pathogenic bacteria as compared to wild-type mice; the hyperplastic response in the colon of transgenic mice encoding NOX1 is significantly increased (see Fig. 4, note specifically the p values shown on Fig. 4 itself). It is further disclosed that these transgenic mice, and cells isolated from these mice, are of use in identifying agents that are of use in treating proliferative disorders, such as colon cancer (for example, see the specification at page 23, line 3 to page 25, line 21). The specification specifically discloses that the mice are of use in methods to identify therapeutic agents, such as by administering the agent to the transgenic animal and analyzing the effectiveness of the agent in the presence of a pathogenic bacterial such as *C. rodentium* (see the specification at page 25, lines 1-16).

The Office action states that as the crypt dept is not significant different between the C57BL/6J transgenic mice expressing NOX1 and in Min (multiple intestinal neoplasia) mice expressing the NOX 1 transgene ("Cre/Nox1/Min" mice), the mice cannot have a utility. Applicants respectfully disagree with the assertions made in the Office action. As discussed above, the data on crypt depth presented in Fig. 4 show differences between transgenic mice heterozygous for a NOX1 transgene and wild-type mice. No data on Cre/Nox1/Min is presented in Fig. 4 (this figure shows that mice harboring a transgene encoding NOX 1 have an increased hyperplastic response in the colon in response to a pathogenic bacteria as compared to wild-type mice). Thus, transgenic mice expressing NOX1 have a substantial, specific and credible utility.

Reconsideration and withdrawal of the rejection is respectfully requested.

*Rejections Under 35 U.S.C. § 112, first paragraph*

Claims 1-6 and 9-18 were rejected as allegedly the claims are not supported by a specific or substantial utility. Claims 2, 5, and 7-9 are canceled herein, rending this rejection moot as applied to these clams. Applicants respectfully disagree with this rejection as applied to the amended claims or as may be applied to the newly added claims.

The Office action states that one of skill in the art could not know how to use the claimed invention as there is no asserted utility. Applicants respectfully disagree with this assertion. In view of the utility clearly asserted in the specification, the amendments of the claims, and further in view of the arguments presented above, Applicants believe that this rejection is rendered moot.

Claims 1-6 and 9-18 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly not being enabled by the specification. Claims 2, 5, and 7-9 are canceled herein, rending this rejection moot as applied to these clams. Applicants respectfully disagree with this rejection as applied to the amended claims or as may be applied to the newly added claims.

The Office action asserts that the specification is not enabling for any non-human transgenic animal. Applicants respectfully disagree with the position of the U.S. Patent and Trademark Office (PTO) that the production of a transgenic animal can only be enabled by an actual experimental showing that such an animal can be produced. However, to advance the prosecution of the present application, the claims are amended herein to be limited to transgenic mice.

Furthermore, the Office action asserts that the specification is only enabling for transgenic mice encoding NOX1, and not any NADPH oxidase, and the production of animals, and effects of expression of different transgenes, is unpredictable. Again, Applicants respectfully disagree with the position of the U.S. PTO that the production of transgenic mice is unpredictable. However, to advance the prosecution of the present application, the claims are amended to be limited to transgenic mice comprising a nucleic acid encoding NOX1.

The Office action asserts that the specification is not enabling as there is no phenotype associated with mice comprising a NOX1 transgene, thus one of skill in the art would not know how to use these animals. Applicants respectfully disagree with this assertion.

As disclosed, for example, in Example 10, Example 11 (see also the specification at pages 31-32) and Fig. 4, mice heterozygous for a transgene encoding NOX 1 have an increased hyperplastic response in the colon in response to a pathogenic bacteria as compared to wild-type mice; the hyperplastic response in the colon of transgenic mice encoding NOX1 is significantly

increased (see Fig. 4, note specifically the p values shown on the figure itself). It is further disclosed that these transgenic mice, and cells isolated from these mice, are of use in identifying agents that are of use in treating proliferative disorders, such as colon cancer (for example, see the specification at page 23, line 3 to page 25, line 21). Thus, mice comprising a NOX1 transgene and their use are fully enabled by the specification.

With regard to the promoter, Applicants submit that the production of transgenic mice, and the use of any promoter, is enabled by the specification. Expression vectors for the production of transgenic mice and promoters of use are described in the specification, for example at page 12, line 25 to page 16, line 2. Moreover, one of skill in the art can readily identify promoters of use. For example, attached as Exhibit A is a printout presentation from M.D. Anderson Cancer Center (available on the internet at http://sciencepark.mdanderson.org/surp/seminars/Transgenic_mice.pdf), which lists promoters known to be of use in the creation of transgenic models (which can be used to produce cancer models). Exhibit B is a list of promoters that have been used to create transgenic mice with a single coding sequence (tet, this is available on the internet at http://www.zmg.uni-mainz.de/tetmouse/tet.htm; note that all citations were available in 2003 or earlier).

It should be noted that the Office action cites Capecchi et al. (*Trends in Genetics* 5(3):70-76, 1989) as teaching that "the potential now exists to generate mice of any desired genotype" (see the Office action at page 23, and Capecchi et al. at page 70, paragraph 3). Thus, the Office action provides an admission that one of skill in the art can readily generate transgenic mice including any gene of interest.

The Example section discloses one specific example, namely mice including a NOX1 cDNA operably linked CX1 promoter. To produce these mice, several lines of mice were utilized.

Mice were generated that carry a construct wherein a nucleic acid encoding a marker (enhanced green fluorescent protein, EGFP) flanked by loxP sequences is included between the NOX1 cDNA and the CX1 promoter. Thus, the construct is as follows:

| | | | | |
|---|---|---|---|---|
| CX1 | LoxP | EGFP | LoxP | NOX1 |

These mice were bred to mice expressing a Cre transgene:

| | |
|---|---|
| Promoter | Cre |

Once these mice are bred with each other, Cre is expressed from its promoter. Cre then acts at the LoxP site; the intervening sequences (EGFP) are excised. The resultant mice then carry following gene:

| | |
|---|---|
| CX1 | NOX1 |

For the Examiner's convenience, a complete description of the use of Cre/Lox in producing transgenic mice (available on the internet at http://bioteach.ubc.ca/MolecularBiology/TargetingYourDNAWithTheCreloxSystem/) is attached as Exhibit C. The Office action alleges

(see page 14, first paragraph) that "an artisan would not be able to predict whether a transgenic mouse could be produced using a CX1 promoter." Applicants respectfully disagree, as not only does the specification describe these animals, but actual working examples are described.

Thus, adequate guidance and working examples are provided by the specification. Accordingly, Applicants submit that the specification is fully enabling for transgenic mice including a promoter operably linked to a NOX1 gene, and the use of these mice.

The Office action further alleges that methods for identifying a therapeutic agent for use in treating inflammation (and colon hyperplasia) are not enabled by the specification, as no phenotype is provided. The Office action makes this assertion based on the erroneous conclusion that Fig. 4 shows a comparison of crypt depth in NOX1 mice and NOX1/Min mice, and that the challenge with a pathogenic bacteria does not result in any change in crypt depth in a NOX1 mouse as compared to a control. The Office action appears to assert that as the mice did not exhibit any difference in inflammation or hyperplasia, they could not be of use in screening for therapeutic agents. Applicants respectfully disagree. The hyperplastic response in the colon of transgenic mice encoding NOX1 is significantly increased as compared to wild-type animals (see Fig. 4, note specifically the p value shown between column 3 and column 4 of Fig. 4).

The claims are amended to be directed to transgenic mice harboring a transgene that includes a nucleic acid sequence set forth as SEQ ID NO: 1 or a degenerate variant thereof operatively linked to a promoter. Applicants respectfully point out that the data presented in the specification clearly documents a specific phenotype in mice expressing NOX1 as compared to control animals.

Mice expressing NOX1 operably linked to a CX1 promoter exhibit an increased overgrowth of colonic epithelial cells upon exposure to pathogenic bacteria. As disclosed in Example 10, Example 11, and Fig. 4 (see the specification at pages 31-32), these mice heterozygous for a transgene encoding NOX 1 have an increased hyperplastic response in the colon in response to pathogenic bacteria as compared to wild-type mice; the hyperplastic response in the colon of transgenic mice encoding NOX1 is significantly increased (see Fig. 4, note specifically the p values shown on the figure itself). It is further disclosed that these transgenic mice, and cells isolated from these mice, are of use in identifying agents that are of use in treating proliferative disorders, such as colon cancer (for example, see the specification at page 23, line 3 to page 25, line 21). The specification specifically discloses that the mice are of use in methods to identify therapeutic agents, such as administering the agent to the transgenic animal and analyzing the effectiveness of the agent in the presence of pathogenic bacteria such as *C. rodentium* (see the specification at page 25, lines 1-16).

The Office action again asserts that as the crypt depth is not significantly different between the C57BL/6J transgenic mice expressing NOX1 and in Min (multiple intestinal neoplasia) mice expressing the NOX 1 transgene ("Cre/Nox1/Min" mice). As discussed above, the data on crypt depth presented in Fig. 4 show differences between transgenic mice heterozygous for a NOX1 transgene and wild-type mice. No data on Cre/Nox1/Min is presented in Fig. 4; this figure shows that mice harboring a transgene encoding NOX 1 have an increased hyperplastic response in the colon in response to pathogenic bacteria as compared to wild-type mice. Reconsideration and withdrawal of the rejection are respectfully requested.

Claims 1-6 and 9-18 were rejected as allegedly there is insufficient written description for any non-human transgenic animal comprising any transgene encoding a NOX or a DUOX. Claims 2, 5, and 7-9 are canceled herein, rendering this rejection moot as applied to these clams. Applicants respectfully disagree with this rejection as applied to the amended claims or as may be applied to the newly added claims.

Solely to advance prosecution, the claims have been limited to mice comprising a transgene comprising a nucleic acid sequence set forth as SEQ ID NO: 1 or a degenerate variant thereof operatively linked to a promoter, wherein the mouse exhibits an increased overgrowth of colonic epithelial cells upon exposure to pathogenic bacteria (claim 1 and dependent claims thereof). The Office action notes that these mice are fully described (see page 17, second full paragraph, although the phenotype of these mice is incorrectly characterized, see the discussion above).

The claimed mice and their use are fully described in the specification; indeed the Office action documents the support for the animals. In addition, as discussed above, a phenotype is clearly disclosed for the claimed transgenic mice. As such, Applicants submit that the specification provides sufficient written description for claims 1, 3-4, 6, 9-18, and the newly added claims. Reconsideration and withdrawal of the rejection are respectfully requested.

Claim 15 is rejected as allegedly the term "inflammation" is not clear and defined. In making this rejection, the Office action asserts that the term cannot be clear because the specification does not disclose what is encompassed by the term.

Applicants submit that one of skill in the art need not define every term of use for the term to be considered "clear" and "definite." The MPEP at 2173.02 states that the terms of the

claim must meet "the threshold requirement of clarity and precision...he or she should allow claims which define the subject matter with a reasonable degree of particularity and definiteness." This should be viewed as to whether "the claim apprises one of ordinary skill in the art of its scope."

Applicants submit that one of ordinary skill in the art can readily understand the term "inflammation;" indeed this term is found is most dictionaries. For example, Merriam-Webster on-line dictionary defines inflammation as: "a local response to cellular injury that is marked by capillary dilatation, leukocytic infiltration, redness, heat, and pain and that serves as a mechanism initiating the elimination of noxious agents and of damaged tissue." Similarly, the American Heritage on-line dictionary defines inflammation as "a localized protective reaction of tissue to irritation, injury, or infection, characterized by pain, redness, swelling, and sometimes loss of function." The on-line medical dictionary defines inflammation as "a localized protective response elicited by injury or destruction of tissues, which serves to destroy, dilute or wall off (sequester) both the injurious agent and the injured tissue. It is characterized in the acute form by the classical signs of pain (*dolor*), heat (*calor*), redness (*rubor*), swelling (*tumour*) and loss of function (*functio laesa*)." For the Examiner's convenience, print-outs of each definition from these on-line dictionaries are attached as Exhibit D.

Applicants submit that the term "inflammation" is clear and definite. Reconsideration and withdrawal of the rejection is respectfully requested.

*Rejections Under 35 U.S.C. § 103*

Claims 1-6 and 9 were rejected as allegedly being obvious over Suh et al. in view of Capecchi et al. Claims 1-4 and 9 were rejected over Dupuy et al. in view of Capecchi et al.

Applicants respectfully disagree with these rejections as applied to the claims as amended, or as may be applied to the newly added claims.

Suh et al. teaches the cloning of a mox1, which has 99.8% identity to SEQ ID NO: 1. and teaches that this gene may play a role in disorders such as cancer and atherosclerosis. Suh et al. does not suggest making transgenic mice, nor does it suggest that transgenic mice expressing NOX1 will exhibit an increased overgrowth of colonic epithelial cells upon exposure to pathogenic bacteria.

Dupuy et al. discloses the purification of pocine and human Duox, and describes that this gene is expressed in the thyroid. Dupuy et al. does not suggest making transgenic mice, let alone transgenic mice expressing NOX1, nor does it provide any suggestion that transgenic mice expressing NOX1 will exhibit an increased overgrowth of colonic epithelial cells upon exposure to pathogenic bacteria.

Capecchi et al. teaches that transgenic mice can be generated of any desired genotype. Capecchi et al. does not discuss NOX1, mice including a NOX1 transgene, or the phenotype of NOX 1 mice (increased overgrowth of colonic epithelial cells upon exposure to pathogenic bacteria).

The legal standard applicable to determinations under 35 U.S.C. § 103 based on a combination of references was reiterated by the Court of Appeals for the Federal Circuit in *In re Dow Chemical Co.*, 837 F.26 469, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988):

> The consistent criterion for the determination of obviousness is whether the prior art would suggest to one of ordinary skill in the art that **this process** [i.e. the process of the invention] shall be carried out and would have **a reasonable expectation of success**, viewed in the light of the prior art. Both the suggestion and the expectation of success

must be found in the prior art, **not in the applicant's disclosure** [emphasis added, citation omitted].

Thus, three elements must be established by the rejection to make a *prima facie* case of obviousness. First the prior art must suggest, or provide an incentive for the combination of references; second, the combination as suggested or motivated by the art must yield the process or invention claimed; and third, the prior art must provide a reasonable expectation of success of the claimed invention. At no point may the applicant's disclosure be used to satisfy one or more of these elements. If any of these elements is absent, the rejection based on obviousness is unsupported.

Against this background, Applicants submit that no *prima facie* case of obviousness has been established. Nothing in Suh et al., Dupuy et al. or Capecchi et al. provides a motive for a combination with the other references. Even if one combined and modified the prior art teachings, there is no suggestion in any of the references that increased overgrowth of colonic epithelial cells upon exposure to pathogenic bacteria would result in mice that carry a NOX1 transgene. Furthermore, there is no suggestion in any of the cited references to teach or suggest the use of the Min background in order to obtain mice with colon cancer. Finally, even if one combined and modified the teachings of Suh et al., Dupuy et al. and/or Capecchi et al., there is no suggestion in any of the references that the claimed mice and their use would have a reasonable expectation of success.

Reconsideration and withdrawal of the rejections are respectfully requested.

*New Power Of Attorney and Revocation of Prior Powers*

*Request for a Telephone Conference*

A New Power of Attorney (POA) has been submitted for the above-referenced application. Applicants respectfully request that the Examiner contact the undersigned for a telephone conference. In addition, if any matters remain to be addressed before a Notice of Allowance is issued, the Examiner is respectfully requested to contact the undersigned at the telephone number listed below.

Respectfully submitted,

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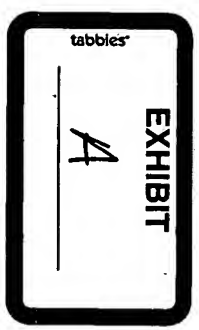
# Transgenic Mice

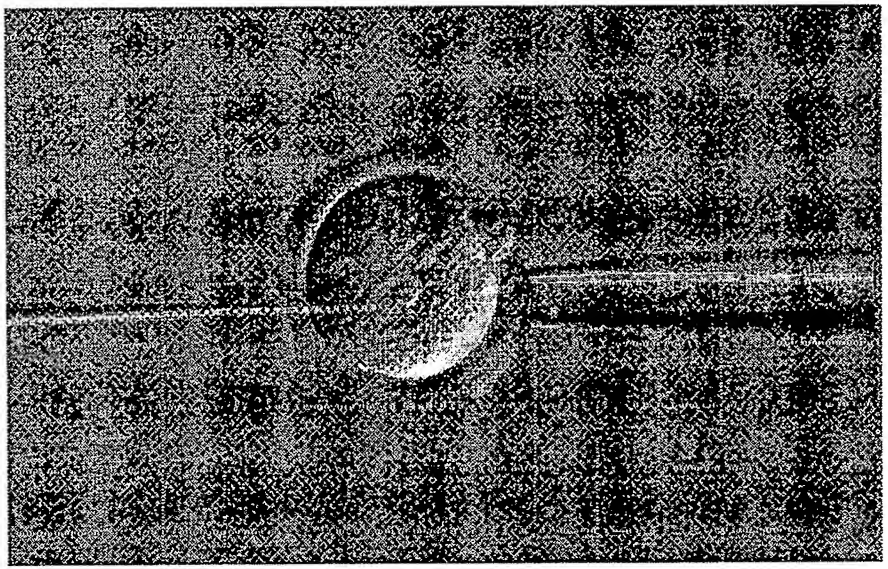
Joyce E. Rundhaug, Ph.D.

M.D. Anderson Cancer Center,
Science Park - Research
Division

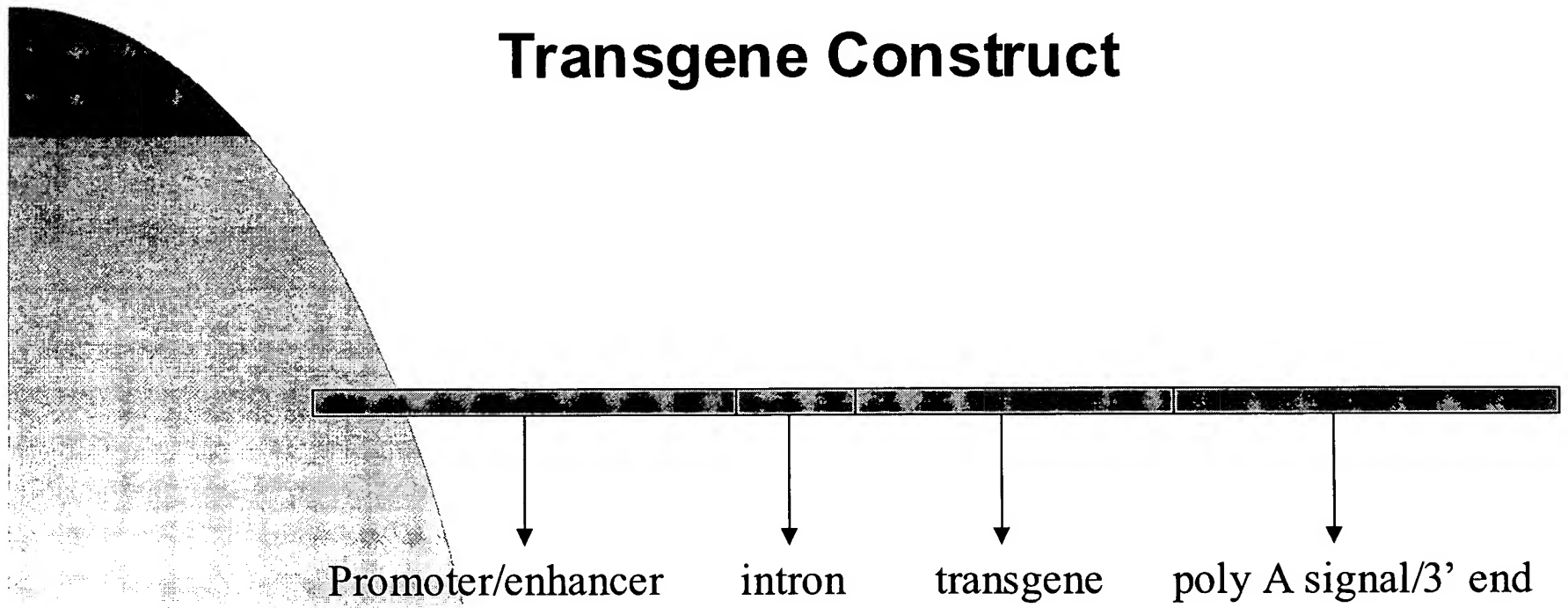


# Creation of transgenic mice

- Transgenic mice are mice in which additional gene(s) have been introduced via injection of DNA into the pronuclei of fertilized eggs.
- The injected eggs are then implanted into surrogate mothers.
- Pups born to the surrogate mothers are then screened for the presence of the transgene in genomic DNA (usually obtained from tail snips) by PCR and/or Southern analyses.



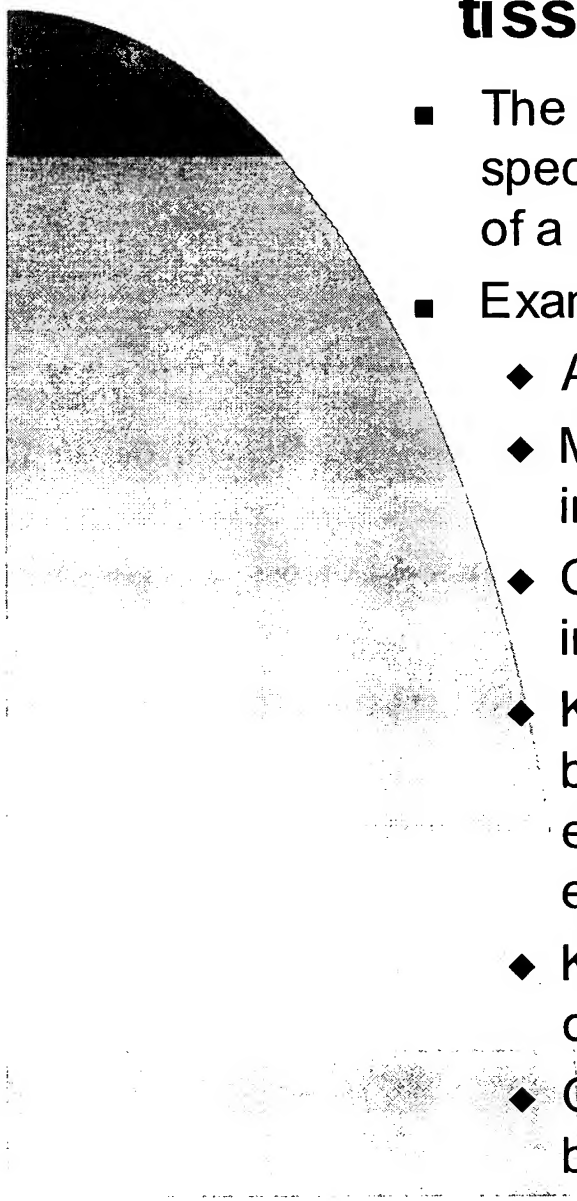
# Transgene Construct



# Reasons for creating transgenic mice

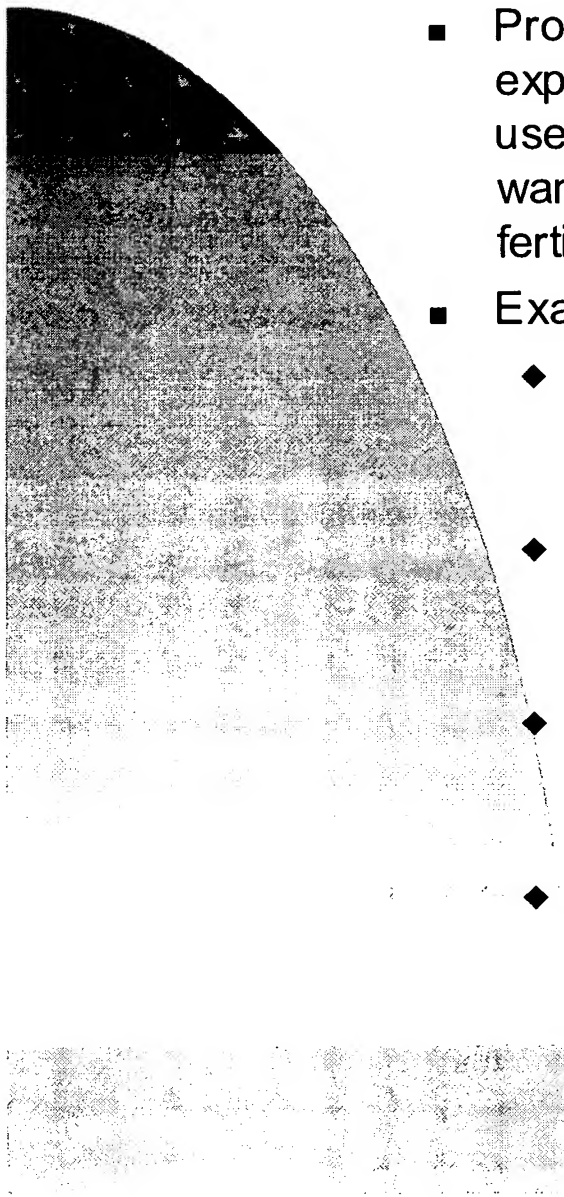
- To express a foreign gene in a specific tissue in order to study its function.
- To overexpress an endogenous gene in a particular tissue in order to study the effects or the contribution of overexpression to a particular cellular or disease process.
- To decrease expression of an endogenous gene in a particular tissue using an antisense construct or to block the function of a particular protein by overexpression of a dominant negative form of that gene in order to study the effects of lack of or decreased expression on normal and/or disease processes.
- To follow expression of particular gene/promoter during embryogenesis and/or development using a reporter gene (e.g., green fluorescent protein, β-galactosidase).

# Targeting the transgene to particular tissue(s)



- The transgene can be targeted to be expressed in specific tissues by using the promoter/enhancer region of a gene known to be expressed only in that tissue.
- Examples of promoters used in transgenic mice:
  - Albumin for expression in the liver
  - Mouse mammary tumor virus (MMTV) for expression in the mammary glands
  - Casein or whey acidic protein (WAP) for expression in lactating mammary glands
  - Keratin-5 or -14 (K5 or K14) for expression in the basal cells and keratin-1 or -10 (K1 or K10) for expression in the suprabasal cells of stratified epithelia (skin, tongue, esophagus, prostate, etc.)
  - Keratin-6 (K6) for expression in hair follicle epithelial cells
  - Glial fibrillary acidic protein (GFAP) for expression in brain astrocytes

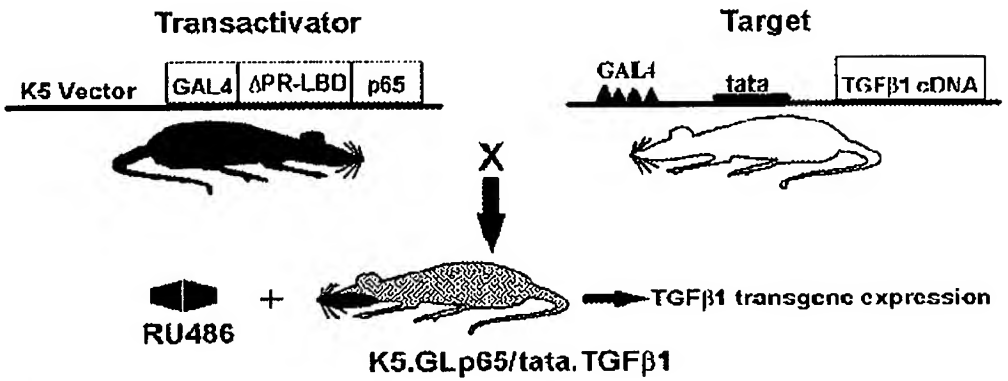
# Use of inducible promoters



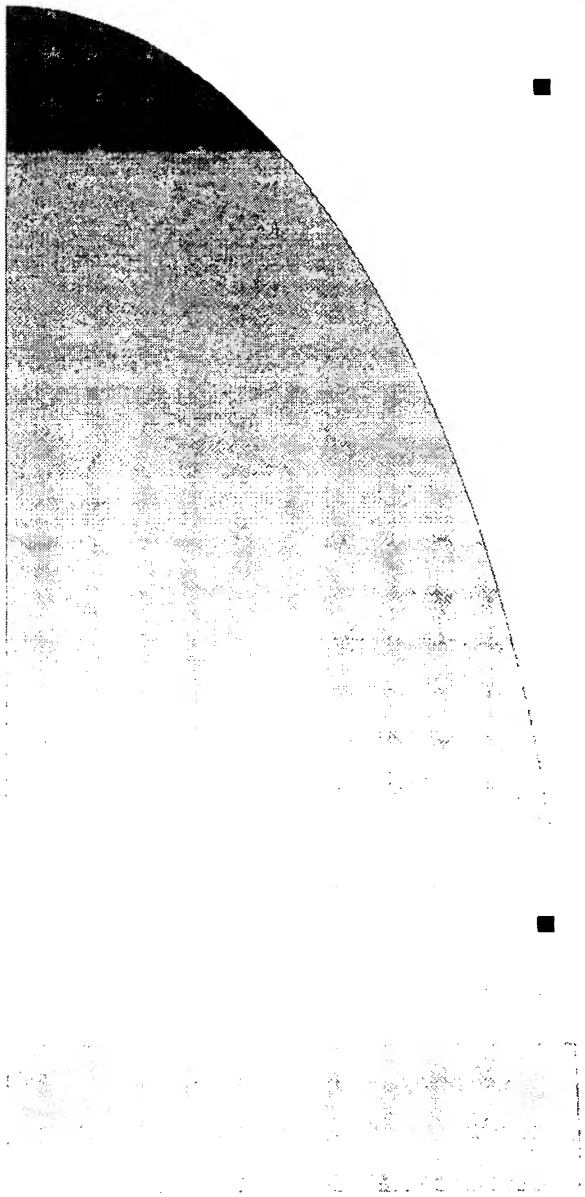
- Promoter/enhancer sequences which are not normally expressed, but with which expression can be induced are used when constitutive expression of the transgene is not wanted in order to prevent developmental and/or fertility/reproductive problems.
- Examples of some inducible promoters:
  - Metallothionein promoter can be induced (primarily in the pancreas, liver, colon, and small intestine) by providing $Zn^{2+}$ in drinking water.
  - Keratin-6 (K6) can be induced to be expressed in interfollicular epidermis by treatments to the skin which induce hyperproliferation.
  - Tetracycline-inducible or- repressible promoters can allow transgene expression to be turned on or off, respectively, by including tetracycline in the drinking water.
  - Bacterial *lac* operator-repressor system uses expression of the *lac* repressor to keep a transgene with *lac* operators in its promoter turned off. Treatment with the lactose analog IPTG in the water turns on transgene expression.

# Gene-Switch Transgenic Mice

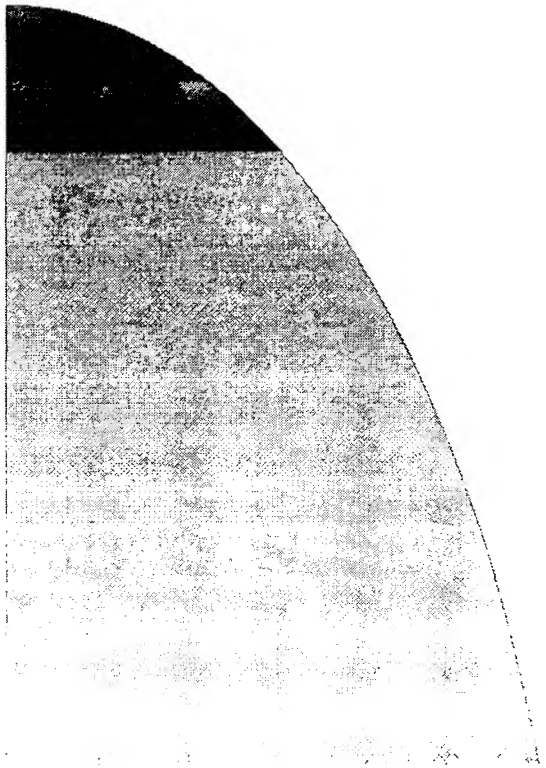
- Like the tet-off/tet-on and lac operator/repressor systems, this system uses 2 transgenic lines:
- 1) A tissue-specific promoter directs expression of a GLVPc transactivator consisting of a fusion molecule of the yeast GAL4 DNA binding domain, a truncated progesterone receptor (which binds progesterone antagonists, but not progesterone), and herpes simplex VP16 transactivation domain.
- 2) A thymidine kinase promoter with 4 copies of the consensus GAL4 binding site drives the expression of the inducible transgene.
- Expression of the transgene in bigenic mice (carry both transgenes) is induced by treatment with progesterone antagonists that bind to the truncated progesterone receptor fusion protein leading to activation of transcription of genes with GAL4 binding sites (only the transgene since GAL4 binding sites are not present in mammalian gene promoters).



# Transgenic mice generated at Science Park



- Using keratin-5 promoter:
  - cyclin D1, D2, and D3
  - Cdk4
  - myc
  - E2F1, E2F2, E2F4, DP1
  - Stat3
  - ATF3
  - IGF1
  - erbB2
  - 8-lipoxygenase, 12-lipoxygenase
  - cyclooxygenase-2
  - Akt, dominant negative Akt
  - Src
  - Cre recombinase
- Using loricrin promoter (targets to primarily skin):
  - 8-lipoxygenase
  - Gelatinase B
  - Truncated type I TGFβ receptor



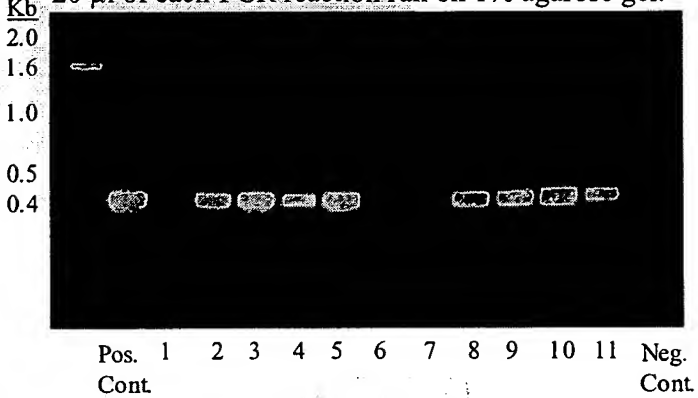
# Transgenic mice generated at Science Park (cont'd)

- Using truncated calbindin9K promoter (targets to smooth muscle cells of uterus/myometrium):
  - HMGIC
  - HMGIY
- Using murine leukemia virus (MuLV) long terminal repeat (LTR) as promoter:
  - MuLV envelope gene
- GFAP promoter (targets to astrocytes):
  - MuLV envelope gene

# Example of Transgenic Mice Generation

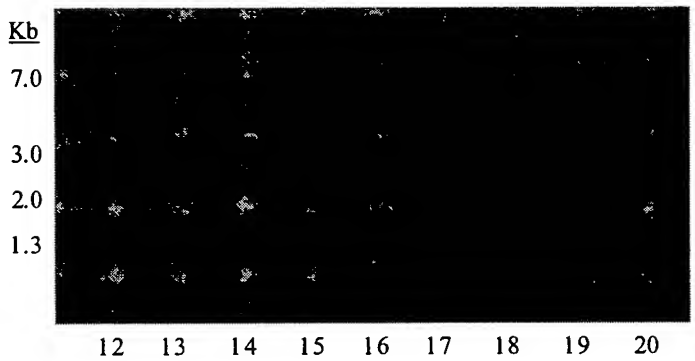

## PCR Example

3 µl of each genomic DNA sample amplified with forward and reverse primers in 50 µl PCR reaction. 20 µl of each PCR reaction run on 1% agarose gel.

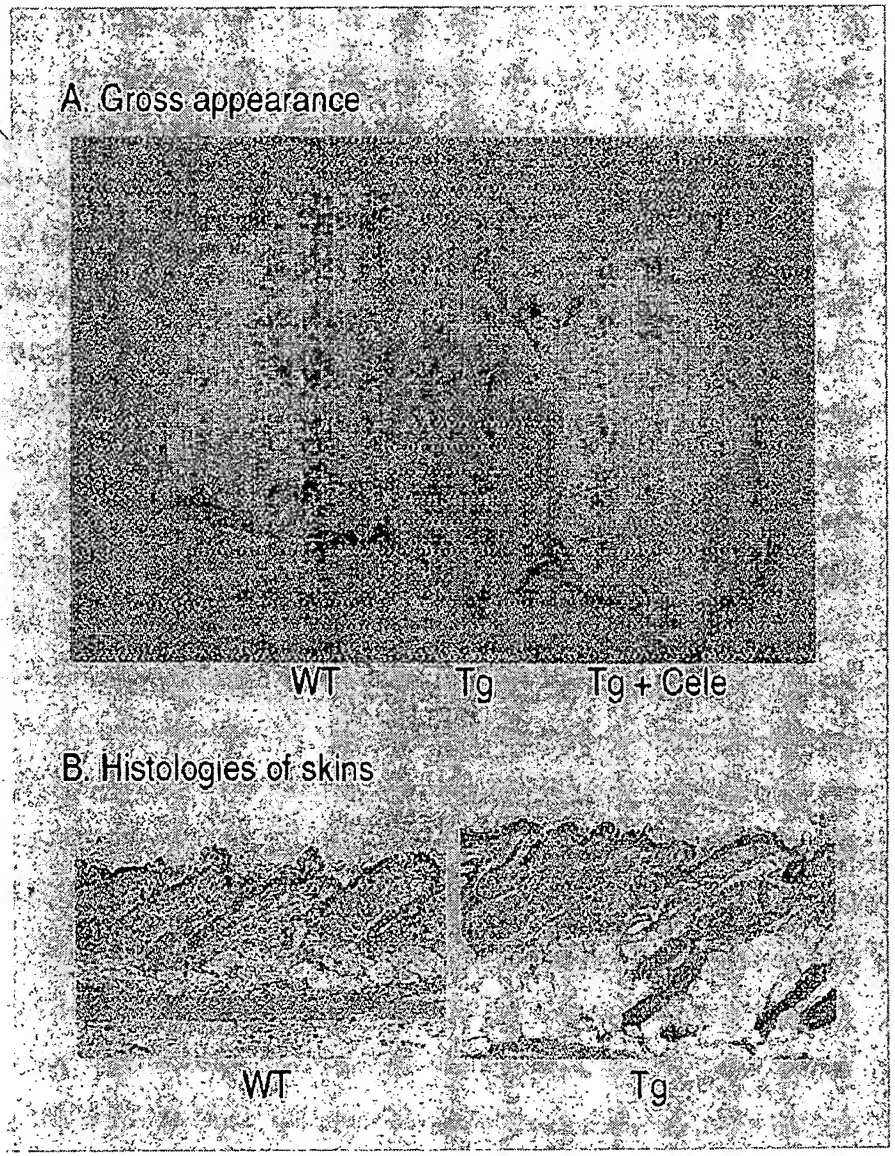



## Southern Blot Example

5 µg genomic DNA from each sample digested with AlwNI, run on 0.7% agarose gel, blotted onto nylon membrane, and probed with $^{32}$P-labeled gelatinase B cDNA probe.



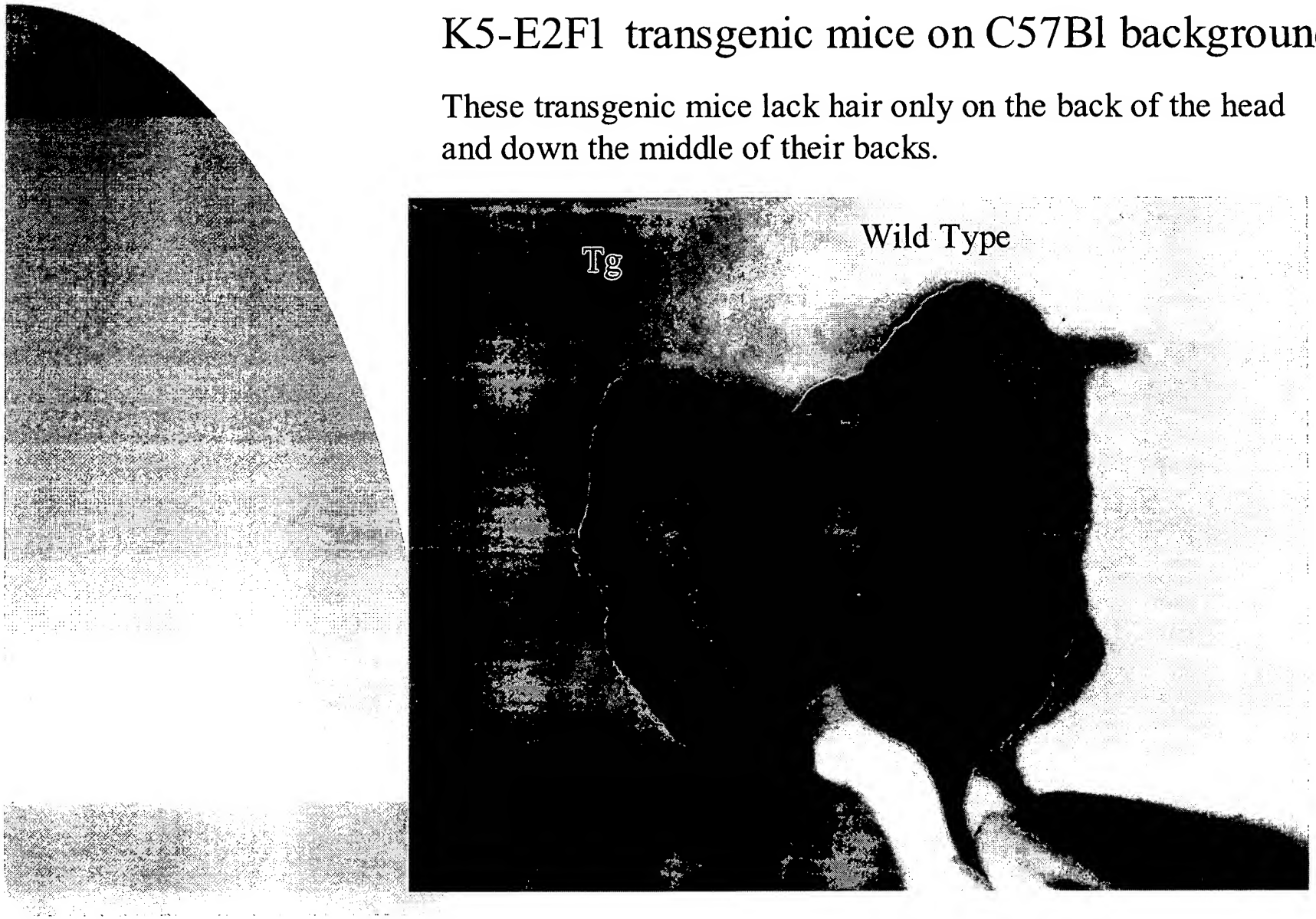
# Keratinl 4-COX-2 transgenic mice




Kl 4-COX-2 transgenic mice have a defect in hair follicle development and so have very little hair. They also have abnormal sebaceous glands. If the mice are treated with a COX-2-specific inhibitor (celecoxib), their hair grows normally and their skin and sebaceous glands appear normal.

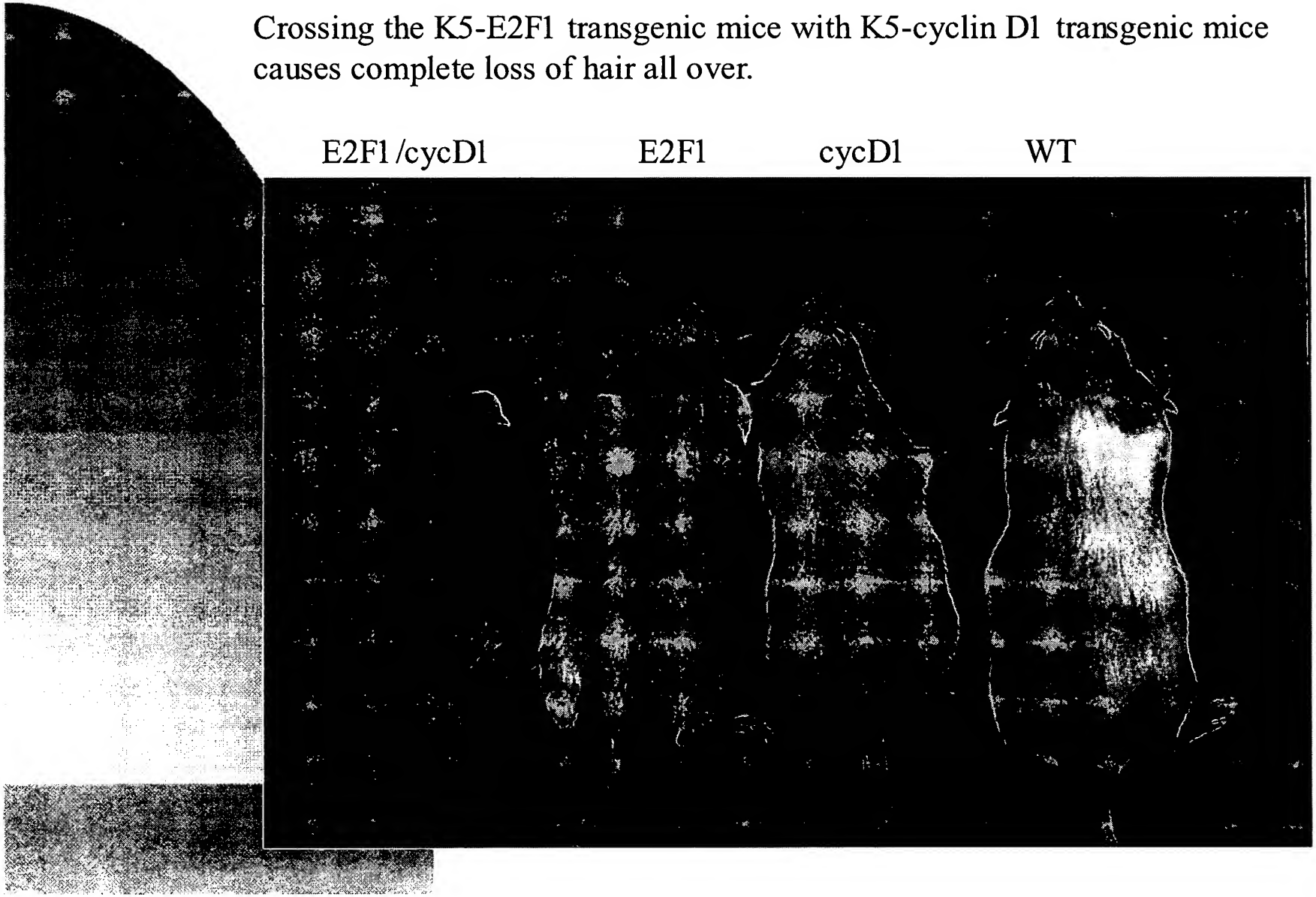
# K5-E2F1 transgenic mice on C57Bl background

These transgenic mice lack hair only on the back of the head and down the middle of their backs.



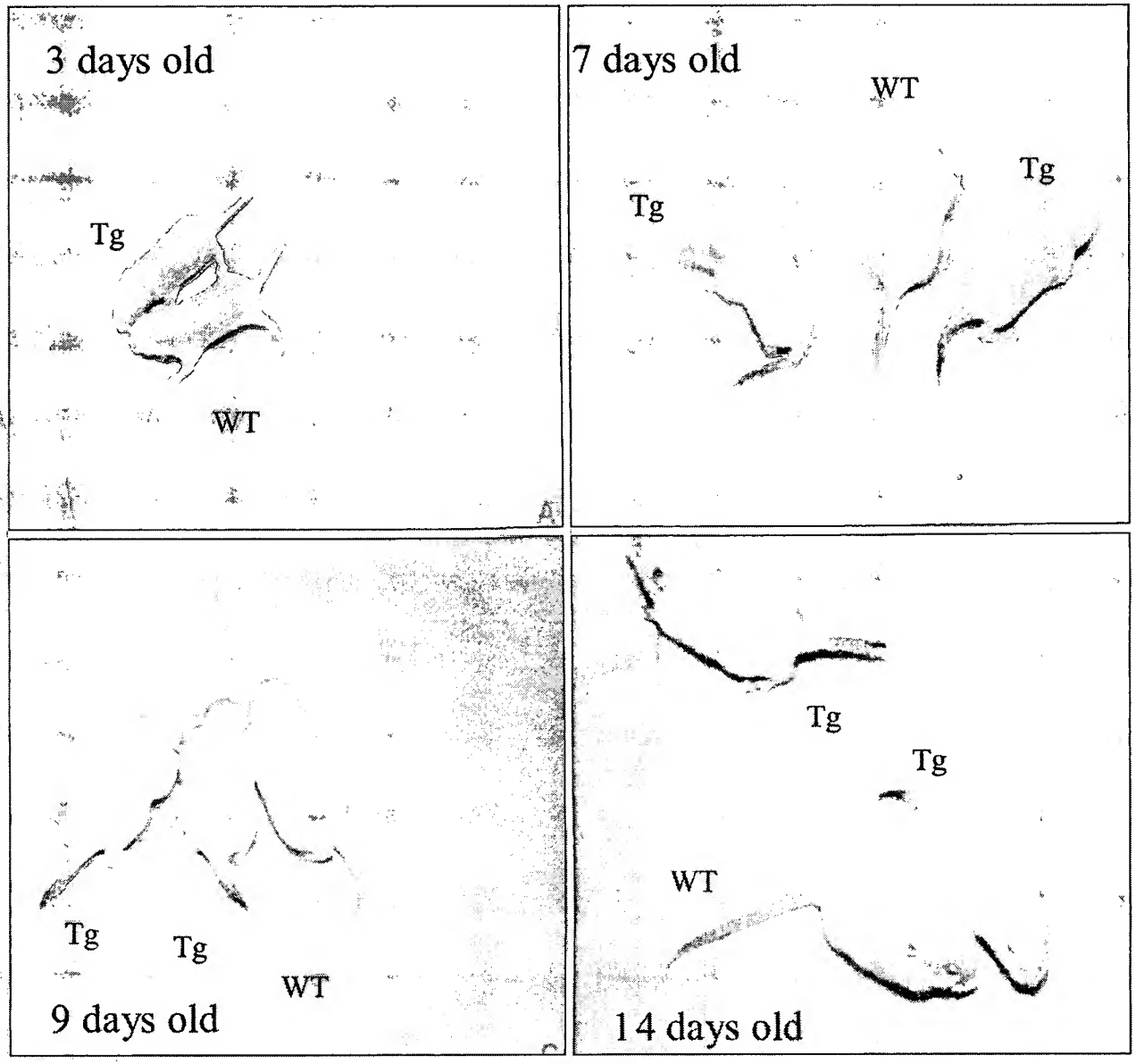
# K5-E2F1 & K5-cyclin D1 transgenic mice

Crossing the K5-E2F1 transgenic mice with K5-cyclin D1 transgenic mice causes complete loss of hair all over.



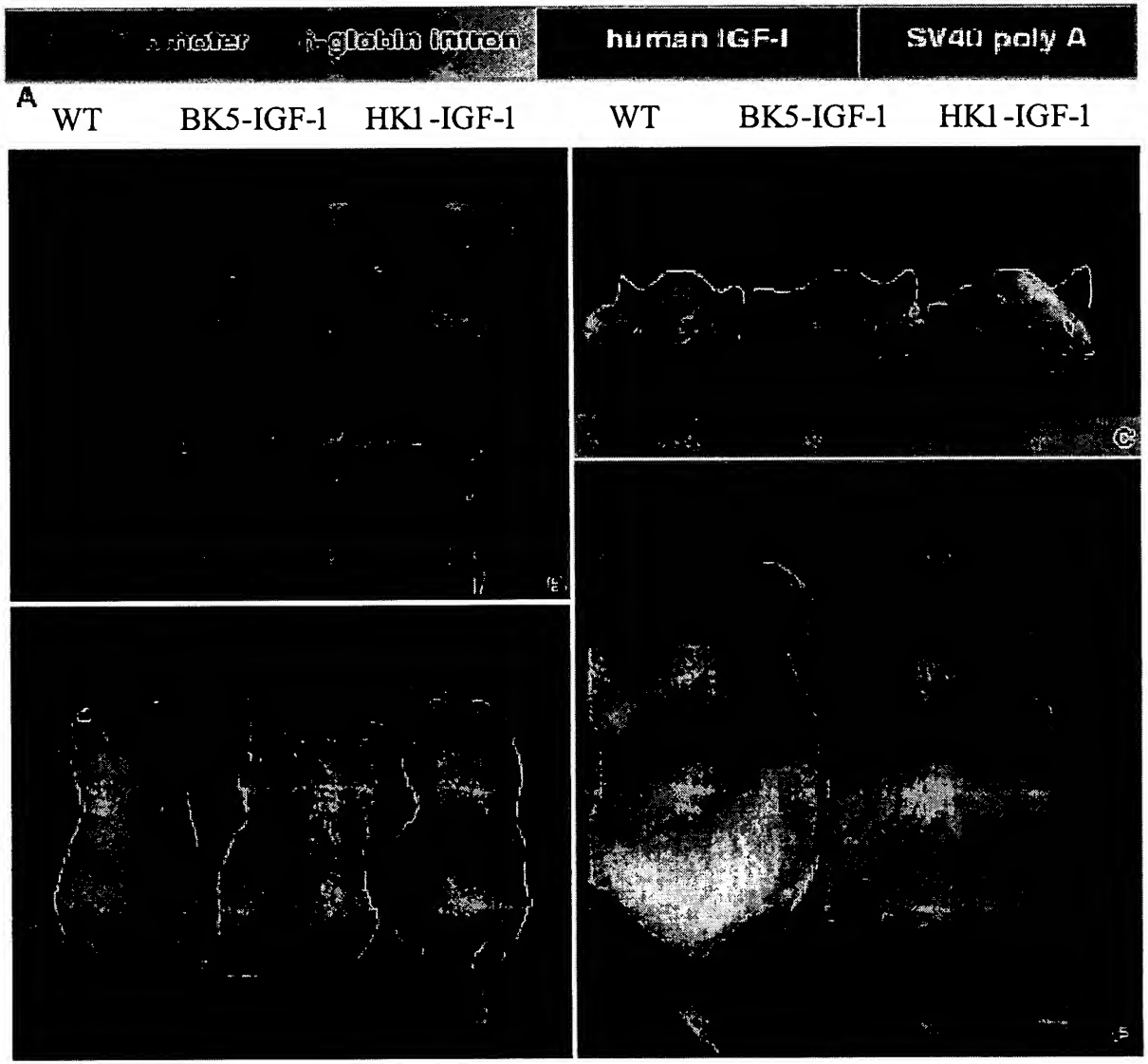
# Loricrin-5' βRI-S transgenic mice

These transgenic mice, which express a truncated TGFβ type I receptor in their skin, develop hemorrhagic and necrotic tails, and are left with short, stubby tails as adults.



# BK5-IGF-1 transgenic mice

Overexpression of IGF-1 in the skin via either the BK5 or HK1 promoter results in mice with extra large ears. The BK5-IGF-1 mice also develop spontaneous skin tumors, as well as prostate lesions.



# Skin Tumorigenesis in Transgenic Mice

The BK5-IGF-1 transgenic mice are more sensitive to chemically-induced skin tumors, as well as developing spontaneous skin tumors.

Table 1 *Responsiveness of BK5.IGF-1 mice to two-stage carcinogenesis*[a]

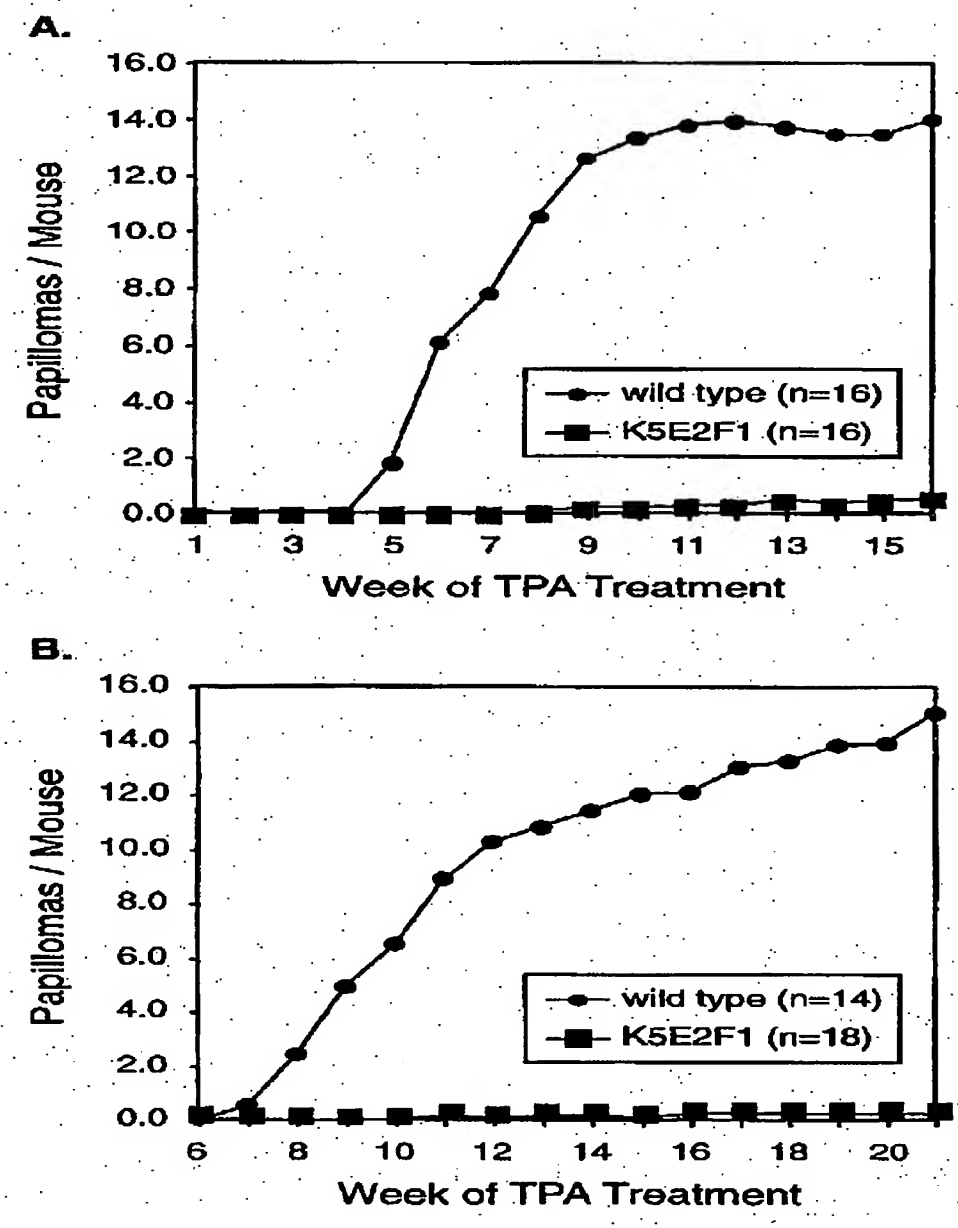
| Initiation | Promotion | % of mice with papillomas | Papillomas/ mouse |
|---|---|---|---|
| Transgenic | | | |
| Acetone (0.2 ml) | TPA (5 nmol) | 72 | 1.00 |
| DMBA (25 nmol) | Acetone (0.2 ml) | 50 | 1.40 |
| DMBA (25 nmol) | TPA (5 nmol) | 100 | 21.00 |
| Nontransgenic | | | |
| Acetone (0.2 ml) | TPA (5 nmol) | 4 | 0.04 |
| DMBA (25 nmol) | Acetone (0.2 ml) | 0 | 0.00 |
| DMBA (25 nmol) | TPA (5 nmol) | 69 | 2.80 |

[a] Groups of 19–27 BK5.IGF-1 transgenic mice and age-matched nontransgenic littermates were initiated with 25 nmol DMBA or received acetone alone (0.2 ml). Two weeks after initiation, promoter treatments began with either 5 nmol TPA or acetone alone twice a week. Promotion was continued for 30 weeks.

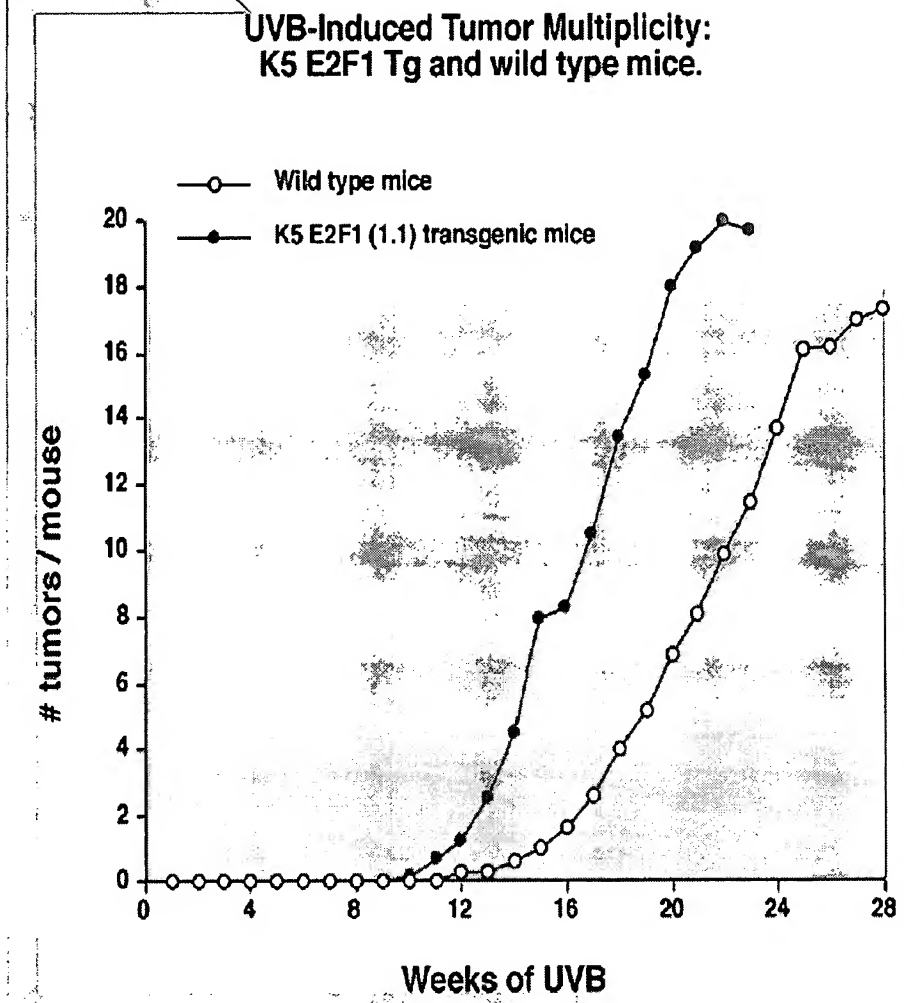
# Skin Tumorigenesis in Transgenic Mice

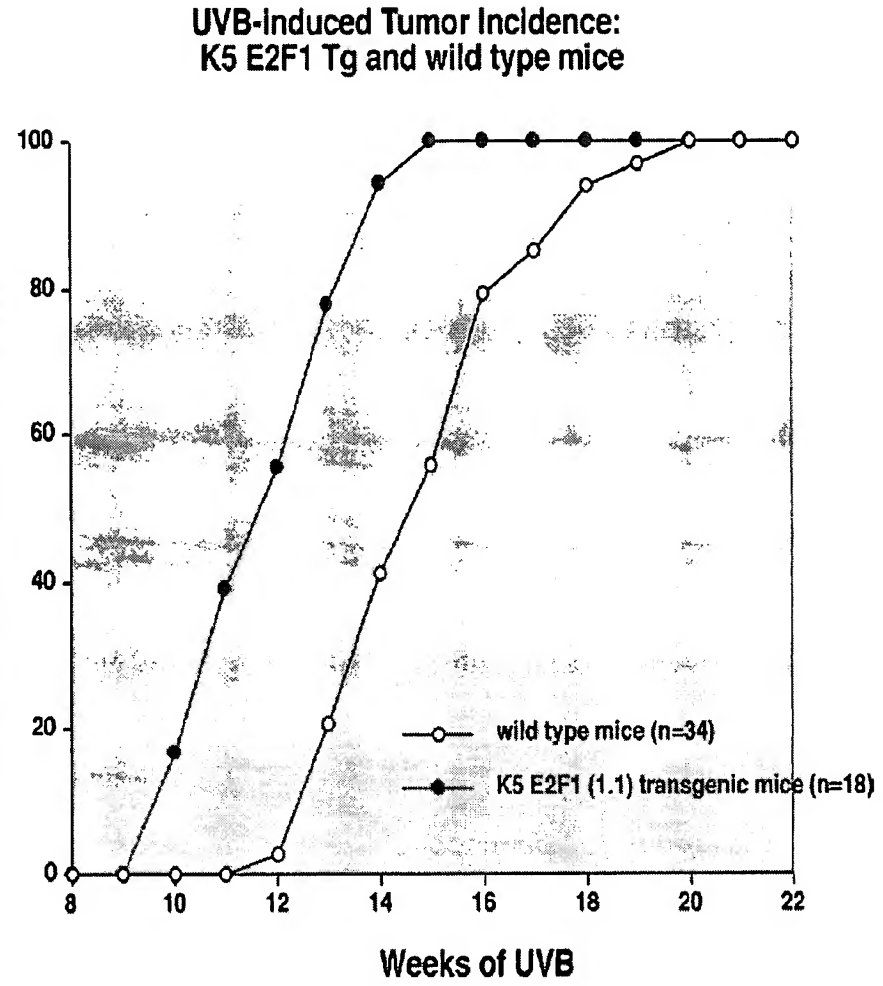
The BK5-E2F1 transgenic mice are resistant to chemically-induced skin tumors, even though they develop some spontaneous skin and oral tumors.

In 1st experiment (Panel A), mice were promoted with 2.5 µg TPA for 6 weeks, then with 0.5 µg TPA until week 16. In the 2nd experiment (Panel B), mice were promoted with 1 µg TPA throughout.



# Ultraviolet (UV)-Induced Skin Tumorigenesis in Transgenic Mice



# General and Tissue-Specific tet-Effector Mouse Lines

[ back ]

| Promoter / Transcription unit | Effector | Technique (tg=transgenic, ki=knock-in) | Tissue Specificity | Reference(s) | |
|---|---|---|---|---|---|
| CMV | tTA rtTA | tg | diverse tissues | Kistner et al., 1996 | h.bujard@zmbh |
| hSP-C (surfactant protein-C) | rtTA | tg | respiratory epithelium | Tichelaar et al., 2000 | jeff.whitset@ch |
| rCcsp (Clara cell secretory protein) | rtTA | tg | respiratory epithelium | Tichelaar et al., 2000 | jeff.whitset@ch |
| rCC10 (Clara cell 10 kDa protein) | rtTA tTS | tg | lung (Clara cells) | Ray et al., 1997 Zhu et al., 2001 | jack.elias@yale |
| bOpsin | rtTA | tg | photoreceptors | Chang et al., 2000 | dzack@bs.jhmi |
| mIRBP (interphotoreceptor retinoid-binding protein) | rtTA | tg | photoreceptors | Chang et al., 2000 | dzack@bs.jhmi |
| αCaMKII | tTA rtTA | tg | brain (neurons of neocortex, hippocampus, amygdala, basal ganglia) | Mayford et al., 1996 Mansuy et al., 1998 | erk5@columbia |
| NSE (neuron specific enolase) | tTA | tg | striatum, cerebellum, CA1-region of hippocampus, neocortex (deep layers) | Chen et al., 1998 | eric.nestler@qn |
| rGH (growth hormone) | rtTA | tg | pituitary gland (somatotropes) | Roh et al., 2001 | kudlow@uab.ec |
| rPRL (prolactin) | tTA | tg | pituitary gland (lactotropes) | Roh et al., 2001 | kudlow@uab.ec |
| PrP (prion protein) | tTA | tg | brain (neocortex, entorhinal cortex, hippocampus, subst. nigra, | Tremblay et al., 1998 | Dept. of Neurol San Francisco, |

EXHIBIT B tabbies

| | | | | | |
|---|---|---|---|---|---|
| | | | thalamus, cerebellum) | | |
| rNestin (tog. with IRES-β-geo) | rtTA | tg | neuroepithelium (tel-, mes-, rhombencephalon, spinal cord, retina, trigeminal ganglion) | Mitsuhashi et al., 2001 | reeves@helix.n |
| hRB (retinoblastoma) (tog. with tetO-CRE) | rtTA | tg | retinal ganglion layer neurons, cerebellar Purkinje cells, thalamus, myocytes of thigh muscles | Utomo et al., 1999 | leew@uthscsa. |
| hPmp22 (peripheral myelin protein 22) | tTA | tg (YAC) | Schwann cells | Perea et al., 2001 | c.huxley@ic.ac. |
| mip2 (proinsulin gene II promoter) (tog. with tetO-PDX-1as) | rtTA | tg | pancreatic β-cells | Lottmann et al., 2001 | rwalther@mail.i |
| rip (rat insuline II promoter) | rtTA | tg | pancreatic β-cells | Thomas et al., 2001 | jhabener@partn |
| mAlb (albumin) | tTA | tg | liver | Manickan et al., 2001 | JakeL@bdg10.i |
| mMup (major urinary protein) | tTA | tg | liver | Manickan et al., 2001 | JakeL@bdg10.i |
| rLAP (liver enriched activator protein) | tTA | tg | liver | Kistner et al., 1996 | h.bujard@zmbh |
| bk5 (keratin 5) | tTA rtTA | tg | skin (epidermal basement layer and hair follicles) | Diamond et al., 2000 | glicka@dc37a.r |
| bK6 (keratin 6) | tTA | tg | skin | Guo et al., 1999 | G.O. Brien, The Research Cente Wynnewood, P/ |
| hK14 (keratin 14) | rtTA | tg | cornified and non-cornified squamous epithelia (skin, esophagus, tongue, cornea) | Xie et al., 1999 | J.E. Kudlow, De Endocrinology, Birmingham, 18 Birmingham, Al |
| hK18 (keratin 18) | rtTA | tg | trachea, bronchi, lungs, submucosal glands | Ye et al., 2001 | jhu@sickkids.o |

| | | | | | |
|---|---|---|---|---|---|
| mTyr (tyrosinase) | rtTA | tg | melanocytes | Chin et al., 1999 | ron_depinho@c lynda_chin@df |
| mEdnrb (endothelin receptor B) | tTA rtTA | ki | melanocytes, enteric neurons | Shin et al., 1999 | S.M. Tilghman, Molecular Biolo Princeton, NJ 0 |
| Fabpl$^{4xat-132}$ (fatty acid binding protein) | rtTA | tg | small intestine, cecum, colon, bladder | Saam and Gordon, 1999 | jgordon@molec |
| rWap (whey acidic protein) (tog. with tetO-CRE) | rtTA | tg | mammary epithelial cells, (kidney glomeruli) | Utomo et al., 1999 | leew@uthscsa. |
| MMTV-LTR | tTA | tg | mammary gland, salivary gland, seminal vesicle, Leydig cells, bone marrow (brain, kidney, liver, spleen) | Hennighausen et al., 1995 | paf@georgetow lotharh@nih.go |
| MMTV-LTR | rtTA | tg | mammary gland, salivary gland, seminal vesicle | D'Cruz et al., 2001 Gunther et al., 2002 | chodosh@mail. |
| β-lactoglobulin (tog. with tetO-αlactalbumin) | rtTA | tg | mammary gland | Soulier et al., 1999 | vilotte@biotec.j |
| hMCK (muscle creatine kinase) | tTA | tg | striated muscle | Ghersa et al., 1998 | rob.hooft@sero |
| MCK (muscle creatine kinase) | tTA | tg | striated muscle | Ahmad et al., 2000 | amalf001@mc. |
| rαMHC (alpha myosin heavy chain) | tTA rtTA | tg | cardiac myocytes | Yu et al., 1996 Passman and Fishman, 1994 Valencik and McDonald, 2001 | fishman@aecor mcdonald.john@ |
| SM22α | tTA | tg | vascular smooth muscle cells | Ju et al., 2001 | mr@sickkids.or |
| mTek | tTA | tg | endothel (embryonic) | Sarao et al., 1998 | ddumont@sunr |
| mTie | tTA | tg | endothel (adult) | Sarao et al., 1998 | ddumont@sunr |
| Ig heavy chain- | | | | | |

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|---|---|---|---|---|---|
| enhancer/SRα-promoter | tTA | tg | hematopoietic cells | Felsher et al., 1999 | felsher@itsa.uc |
| Ig heavy chain-enhancer/minimal promoter | tTA | tg | developing B- and T-cells in spleen and thymus (lymphopoiesis); skeletal muscle | Hess et al., 2001 | thomas.wirth@ |
| hCD2 | rtTA | tg | T-cells | Legname et al., 2000 | rzamoys@nimr. |
| Lck | tTA | tg | T-cells | Leenders et al., 2000 | cbdm@joslin.ha |
| MHCIIEα$^{k}$ | tTA | tg | thymic epithelial cells | Witherden et al., 2000 | cbdm@igbmc.u |
| murine SM22 promoter | rtTA | tg | smooth muscle | West et al. 2004 | david.rodman@ |
| Foxg1 genomic locus | tTA | ki | brain (progenitors) | Hanashima et al. 2004 | fishell@saturn. |
| humanCD34 YAC | tTA | tg | haematopoietic stem cells,CMPs and MEPs, megakaryocytes | Huettner et al., 2003 | dtenen@bidmc. |
| surfactant protein C promoter | rtTA | tg | lung | Mucenski et al. 2003 | michael.mucen |
| Clara cell secretory protein promoter | rtTA | tg | lung | Mucenski et al. 2003 | michael.mucen |
| tyrosinase promoter and Locus Control Region (LCR) | rtTA | tg | retina | Lavado et al. 2003 | |
| liver activator protein (LAP) promoter | rtTA | tg | kidney and liver | Gallagher et al. 2003 | ralph.witzgall@ |
| human podocin (NPHS2) gene promoter | rtTA | tg | podocytes in the kidney | Shigehara et al., 2003 | jbkopp@nih.go |
| GABA(A) receptor alpha6 gene promoter | rtTA | tg | cerebellar granule cells of the brain | Yamamoto et al., 2003 | Yamamoto.Mut |
| small-conductance $Ca2^{+}$ activated $K^{+}$ channel | tTA | ki | mesenteric arteries of the endothelium | Taylor et al., 2003 | mtnelson@zoo. |
| Tet- promoter | tTA | tg | various tissues | Hwang et al., 2001 | kimyongkyu@h |

| | | | | | |
|---|---|---|---|---|---|
| rat Clara cell secretory protein 2.3-kb promoter | rtTA | tg | lung | Yan et al., 2003 | Cong.Yan@cch |
| human β-actin promoter | tTA | tg | various tissues | Lamposa et al., 2003 | pg.wells@utoro |
| mouse muscle creatine kinase (MCK) promoter | rtTA | tg | skeletal muscle | Grill et al., 2003 | *pba@email.ariz* |
| major immediate-early human CMV promoter and enhancer | tTA | tg | multiple tissues | Haribhai et al., 2003 | cwilliam@mcw. |
| hCMV enhancer/chicken β-actin promoter | rtTA | tg | multiple tissues but not in haemopoietic organs | Manfra et al., 2003 | sergio.lira@ms: |
| mouse alpha-myosin heavy chain promoter | tTA/rtTA | tg | heart | Sanbe et al., 2003 | jeff.robbins@ch |
| MMTV-LTR | rtTA | tg | mammary gland | Gunther et al., 2003 | chodosh@mail. |
| SM22$\alpha$ promoter | tTA | tg | vascular SMCs of aorta, carotid, mesentery, liver, lung, kidney, and spleen | You et al., 2003 | mansoor.husair |
| murine rhodopsin promoter | tTA | tg | photoreceptor cells of the outer nuclear layer of the retina | Angeletti et al., 2003 | marigo@tigem. |
| rat insulin promoter | tTA | tg | beta-cells of the pancreas | Christen et al., 2002 | matthias@liai.o |
| small-conductance $Ca^{2+}$-activated potassium channel (SK channel) | tTA | tg | magnocellular neurons of the supraoptic nucleus and in many smooth muscles, including the uterus | Bond et al., 2000 | adelman@ohsu |
| Clara cell–specific CC10 promoter | rtTA | tg | lung, airways | Mehrad et al., 2002 | borna.mehrad@ |
| | | | peripheral respiratory epithelial cells in | Clark et al., 2001 | |

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|---|---|---|---|---|---|
| human SP-C promoter | rtTa | tg | the lungs of fetal [postconception (pc) *day 15*] and adult mice | Pearl et al., 2002 | jeff.whitsett@ch |
| rat CCSP promoter | rtTA | tg | tracheobronchial and type II cells | Clark et al., 2001<br>Pearl et al., 2002 | jeff.whitsett@ch |
| tek/Tie2 promoter/intron enhancer | rtTA | tg | endothelial cells | Teng et al., 2002 | jamie.topper@r |
| *Pdx1* gene | tTA | ki | pancreas | Holland et al., 2002 | raymond.macdc |
| immunoglobulin heavy chain enhancer and the SRa promoter (EmSR) | tTA | tg | haematopoietic cells and immature osteoblasts | Jain et al., 2002<br>Felscher et al., 1999 | dfelsher@lelanc |
| neuron-specific enolase (NSE) promoter | tTA | tg | brain | Sakai et al., 2002 | ronald.duman@ |
| CMV enhancer/chicken β-actin promoter | rtTA | tg | high levels of expression in heart; moderate expression levels in skin, kidney, thymus, and lung; and low expression levels in spleen and liver | Wiekowski et al., 2001 | maria.wiekowsk |
| human K14 promoter | tTA | tg | mammary gland | Dunbar et al., 2001 | john.wysolmers |
| mouse albumin promoter/enhancer | tTa | tg | skeletal muscle, heart, liver, lung and spleen | Raben et al., 2001 | rabenn@arb.nia |
| *Lck* proximal promoter | tTA | tg | lymphoid organs (with the exception of a weak signal in the ovaries) | Labrecque et al., 2001 | cb@joslin.harva |
| mouse proinsulin gene II promoter | rtTA | tg | β-cells of pancreatic islets. | Lottmann et al., 2002 | rwalther@mail.u |
| hCMV | tTA/rtTA | tg in ES cells | multiple tissues | Fedorov et al., 2001 | rappur@mail.ur |
| prion protein (PrP) | | | neuronal and glial | Gotz et al., | |

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|---|---|---|---|---|---|
| promoter | tTA | tg | cells | 2001 | goetz@bli.unizh |

last edited by Jens Rudolph on 04/14/04

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[ back ]

| Promoter / Transcription unit | Effector | Technique (tg=transgenic, ki=knock-in) | Tissue Specificity | Reference(s) | |
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| hSP-C (surfactant protein-C) | rtTA | tg | respiratory epithelium | Tichelaar et al., 2000 | jeff.whitset@ch |
| rCcsp (Clara cell secretory protein) | rtTA | tg | respiratory epithelium | Tichelaar et al., 2000 | jeff.whitset@ch |
| rCC10 (Clara cell 10 kDa protein) | rtTA tTS | tg | lung (Clara cells) | Ray et al., 1997 Zhu et al., 2001 | jack.elias@yale |
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| rGH (growth hormone) | rtTA | tg | pituitary gland (somatotropes) | Roh et al., 2001 | kudlow@uab.ec |
| rPRL (prolactin) | tTA | tg | pituitary gland (lactotropes) | Roh et al., 2001 | kudlow@uab.ec |
| PrP (prion protein) | tTA | tg | brain (neocortex, entorhinal cortex, hippocampus, subst. nigra, | Tremblay et al., 1998 | Dept. of Neurol San Francisco, |

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| | | | thalamus, cerebellum) | | |
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| bK6 (keratin 6) | tTA | tg | skin | Guo et al., 1999 | G.O. Brien, The Research Cente Wynnewood, P/ |
| hK14 (keratin 14) | rtTA | tg | cornified and non-cornified squamous epithelia (skin, esophagus, tongue, cornea) | Xie et al., 1999 | J.E. Kudlow, De Endocrinology, Birmingham, 18 Birmingham, AL |
| hK18 (keratin 18) | rtTA | tg | trachea, bronchi, lungs, submucosal glands | Ye et al., 2001 | jhu@sickkids.o |

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|---|---|---|---|---|---|
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| mEdnrb (endothelin receptor B) | tTA rtTA | ki | melanocytes, enteric neurons | Shin et al., 1999 | S.M. Tilghman, Molecular Biolo Princeton, NJ 0 |
| Fabpl$^{4xat-132}$ (fatty acid binding protein) | rtTA | tg | small intestine, cecum, colon, bladder | Saam and Gordon, 1999 | jgordon@molec |
| rWap (whey acidic protein) (tog. with tetO-CRE) | rtTA | tg | mammary epithelial cells, (kidney glomeruli) | Utomo et al., 1999 | leew@uthscsa. |
| MMTV-LTR | tTA | tg | mammary gland, salivary gland, seminal vesicle, Leydig cells, bone marrow (brain, kidney, liver, spleen) | Hennighausen et al., 1995 | paf@georgetow lotharh@nih.go |
| MMTV-LTR | rtTA | tg | mammary gland, salivary gland, seminal vesicle | D'Cruz et al., 2001 Gunther et al., 2002 | chodosh@mail. |
| β-lactoglobulin (tog. with tetO-αlactalbumin) | rtTA | tg | mammary gland | Soulier et al., 1999 | vilotte@biotec.j |
| hMCK (muscle creatine kinase) | tTA | tg | striated muscle | Ghersa et al., 1998 | rob.hooft@sero |
| MCK (muscle creatine kinase) | tTA | tg | striated muscle | Ahmad et al., 2000 | amalf001@mc. |
| rαMHC (alpha myosin heavy chain) | tTA rtTA | tg | cardiac myocytes | Yu et al., 1996 Passman and Fishman, 1994 Valencik and McDonald, 2001 | fishman@aecor mcdonald.john@ |
| SM22α | tTA | tg | vascular smooth muscle cells | Ju et al., 2001 | mr@sickkids.or |
| mTek | tTA | tg | endothel (embryonic) | Sarao et al., 1998 | ddumont@sunr |
| mTie | tTA | tg | endothel (adult) | Sarao et al., 1998 | ddumont@sunr |
| Ig heavy chain- | | | | | |

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|---|---|---|---|---|---|
| enhancer/SRα-promoter | tTA | tg | hematopoietic cells | Felsher et al., 1999 | felsher@itsa.uc |
| Ig heavy chain-enhancer/minimal promoter | tTA | tg | developing B- and T-cells in spleen and thymus (lymphopoiesis); skeletal muscle | Hess et al., 2001 | thomas.wirth@ |
| hCD2 | rtTA | tg | T-cells | Legname et al., 2000 | rzamoys@nimr. |
| Lck | tTA | tg | T-cells | Leenders et al., 2000 | cbdm@joslin.ha |
| MHCIIEα$^{K}$ | tTA | tg | thymic epithelial cells | Witherden et al., 2000 | cbdm@igbmc.u |
| murine SM22 promoter | rtTA | tg | smooth muscle | West et al. 2004 | david.rodman@ |
| Foxg1 genomic locus | tTA | ki | brain (progenitors) | Hanashima et al. 2004 | fishell@saturn.r |
| humanCD34 YAC | tTA | tg | haematopoietic stem cells,CMPs and MEPs, megakaryocytes | Huettner et al., 2003 | dtenen@bidmc. |
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| Clara cell secretory protein promoter | rtTA | tg | lung | Mucenski et al. 2003 | michael.mucens |
| tyrosinase promoter and Locus Control Region (LCR) | rtTA | tg | retina | Lavado et al. 2003 | |
| liver activator protein (LAP) promoter | rtTA | tg | kidney and liver | Gallagher et al. 2003 | ralph.witzgall@ |
| human podocin (NPHS2) gene promoter | rtTA | tg | podocytes in the kidney | Shigehara et al., 2003 | jbkopp@nih.gov |
| GABA(A) receptor alpha6 gene promoter | rtTA | tg | cerebellar granule cells of the brain | Yamamoto et al., 2003 | Yamamoto.Mut |
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| Tet- promoter | tTA | tg | various tissues | Hwang et al., 2001 | kimyongkyu@h |

| | | | | | |
|---|---|---|---|---|---|
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| hCMV enhancer/chicken β-actin promoter | rtTA | tg | multiple tissues but not in haemopoietic organs | Manfra et al., 2003 | sergio.lira@mss |
| mouse alpha-myosin heavy chain promoter | tTA/rtTA | tg | heart | Sanbe et al., 2003 | jeff.robbins@ch |
| MMTV-LTR | rtTA | tg | mammary gland | Gunther et al., 2003 | chodosh@mail. |
| SM22$\alpha$ promoter | tTA | tg | vascular SMCs of aorta, carotid, mesentery, liver, lung, kidney, and spleen | You et al., 2003 | mansoor.husair |
| murine rhodopsin promoter | tTA | tg | photoreceptor cells of the outer nuclear layer of the retina | Angeletti et al., 2003 | marigo@tigem. |
| rat insulin promoter | tTA | tg | beta-cells of the pancreas | Christen et al., 2002 | matthias@liai.o |
| small-conductance $Ca^{2+}$-activated potassium channel (SK channel) | tTA | tg | magnocellular neurons of the supraoptic nucleus and in many smooth muscles, including the uterus | Bond et al., 2000 | adelman@ohsu |
| Clara cell–specific CC10 promoter | rtTA | tg | lung, airways | Mehrad et al., 2002 | borna.mehrad@ |
| | | | peripheral respiratory epithelial cells in | Clark et al., 2001 | |

| | | | | | |
|---|---|---|---|---|---|
| human SP-C promoter | rtTa | tg | the lungs of fetal [postconception (pc) *day 15*] and adult mice | Pearl et al., 2002 | jeff.whitsett@cl |
| rat CCSP promoter | rtTA | tg | tracheobronchial and type II cells | Clark et al., 2001<br>Pearl et al., 2002 | jeff.whitsett@cl |
| tek/Tie2 promoter/intron enhancer | rtTA | tg | endothelial cells | Teng et al., 2002 | jamie.topper@r |
| *Pdx1* gene | tTA | ki | pancreas | Holland et al., 2002 | raymond.macdc |
| immunoglobulin heavy chain enhancer and the SRa promoter (EmSR) | tTA | tg | haematopoietic cells and immature osteoblasts | Jain et al., 2002<br>Felscher et al., 1999 | dfelsher@lelanc |
| neuron-specific enolase (NSE) promoter | tTA | tg | brain | Sakai et al., 2002 | ronald.duman@ |
| CMV enhancer/chicken β-actin promoter | rtTA | tg | high levels of expression in heart; moderate expression levels in skin, kidney, thymus, and lung; and low expression levels in spleen and liver | Wiekowski et al., 2001 | maria.wiekowsk |
| human K14 promoter | tTA | tg | mammary gland | Dunbar et al., 2001 | john.wysolmers |
| mouse albumin promoter/enhancer | tTa | tg | skeletal muscle, heart, liver, lung and spleen | Raben et al., 2001 | rabenn@arb.nia |
| *Lck* proximal promoter | tTA | tg | lymphoid organs (with the exception of a weak signal in the ovaries) | Labrecque et al., 2001 | cb@joslin.harva |
| mouse proinsulin gene II promoter | rtTA | tg | β-cells of pancreatic islets. | Lottmann et al., 2002 | rwalther@mail.i |
| hCMV | tTA/rtTA | tg in ES cells | multiple tissues | Fedorov et al., 2001 | rappur@mail.ur |
| prion protein (PrP) | | | neuronal and glial | Gotz et al., | |

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| promoter | tTA | tg | cells | 2001 | goetz@bli.unizh |

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# Targeting your DNA with the Cre/*lox* System

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Graphics: Jane Wang

It has been 15 years now that the Cre/*lox* system has been used as a way to artificially control gene expression. If your radar hasn't picked it up yet, you're missing out on a clever way to move pieces of DNA around in a cell. Over the years, this system has allowed researchers to create a variety of genetically modified animals and plants with the gene of their choice being externally regulated[1]. This has contributed to our understanding of how individual genes and proteins work.

## How it works

The system begins with the *cre* gene, short for **c**yclization **re**combination, which encodes a site-specific DNA recombinase logically named **Cre**[1,2]. A site-specific DNA recombinase means that the Cre protein can recombine DNA when it locates specific sites in a DNA molecule (see Figure 1). These sites are known as ***loxP*** (locus of X-over P1) sequences, which are 34 base pairs long and magnets for the Cre to recombine the DNA surrounding them[2].

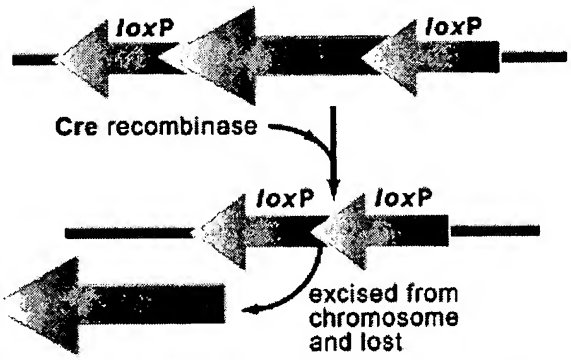


**Figure 1. A model of Cre Function.** The *loxP* sites recognized by Cre are represented with thick arrows and their DNA sequence is shown at the bottom.

When cells that have *loxP* sites in their genome also express Cre, the protein springs into action, catalyzing a reciprocal recombination event between the *loxP* sites (see Figure 1). What does this mean? Well...the double stranded DNA is cut at both *loxP* sites by the Cre protein and then ligated (glued) back together. As a result, the DNA in between the *loxP* sites is excised and subsequently degraded. It is a quick and efficient process.

## Why Cre/*loxP* excision of DNA is so useful

The *loxP* sequence originally comes from the P1 bacteriophage, which is a bacterial virus that, quite reasonably, contains DNA that is not found in animals or plants[1]. Since the *loxP* sequences are also 34 base pairs long there is virtually no chance that you would randomly find them in a genome. Therefore, *loxP* sequences can be artificially inserted into animals or plants and used for the precise excision of DNA, without worrying about cutting other parts of an organism's genome.

## Regulating a Gene Using the Cre/*loxP* System

For a gene to produce a protein it requires a 'promoter.' This is a section of DNA in front of the gene that functions to recruit the cellular machinery that will initiate the multi-step process of protein production (called gene expression). How the promoter functions to do this can vary, from always recruiting cellular machinery and thus always being 'on', to only doing this in specific tissues or cell types, or being inducible and thus only functioning in the presence of a specific factor or condition. Each type will dictate the amount of protein a

gene can produce and thus ultimately control aspects of its function. For this reason, a promoter is considered a 'regulator' of gene function.

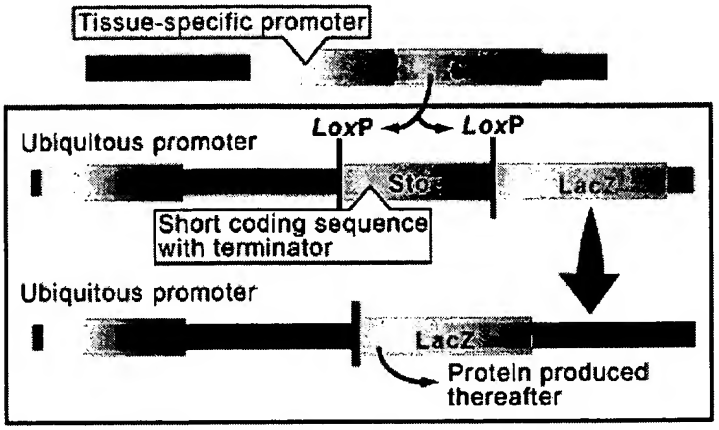


**Figure 2. Cre/lox Regulation of a Protein Expression.** The gene lacZ has *LoxP* sequences containing a stop signal that prevent the gene from being expressed. When exposed to the Cre protein the *LoxP* and stop signal are excised and the gene is expressed. Conditions in which the cre is present thus regulated the expression of the lacZ gene.

An artificial type of this regulation can be achieved with the Cre/lox system[1,3-4]. To do this you need to create a transgenic organism with an always 'on', or ubiquitous, promoter that is attached to and ready to regulate the gene you would like to control. The trick is: in between the promoter and the gene, a 'stop' sequence surrounded with *loxP* sites is inserted (see Figure 2). The stop sequence is a short sequence with several transcriptional stop codons (terminators) that will prevent the gene from producing a protein.

Take the example in Figure 2, where you are trying to control the gene lacZ. Transgenic organisms with a 'stop' sequence between the promoter and gene cannot express the LacZ protein. When Cre is present in the cells of this organism, it catalyzes recombination between the *loxP* sites, thereby deleting the 'stop' sequence. With the stop sequence deleted the organism would express LacZ, or whichever other gene that was being studied in its place. The conditions in which the Cre is present thus regulated the expression of the gene.

## The Cre/lox System in Action

An example of transgenic mice provides an example of how the Cre/lox system can be used. In this case we would like to produce a mouse that no longer has a target gene in only one cell type. The Cre/lox system provides a method to do this[4] (see Figure 3):

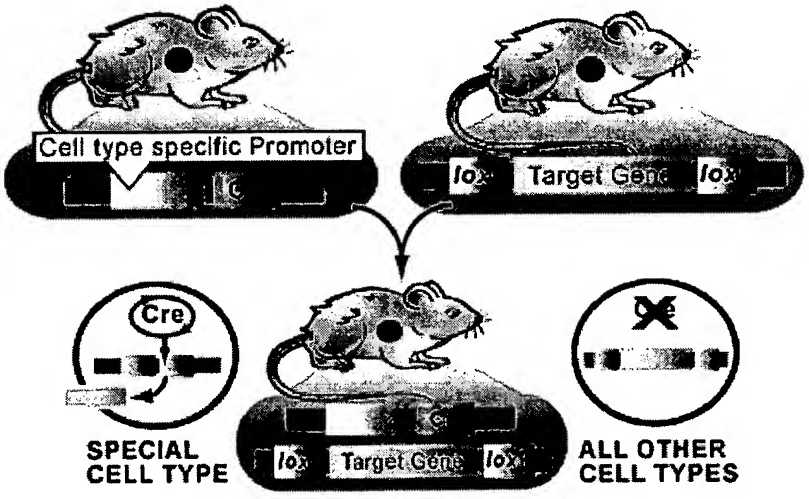


**Figure 3. Cre/*lox* Mouse Breeding.** Mice with the Cre protein expressing in a specific cell type are bred with mice that contain a target gene surrounded by *loxP* sites. When the mice are bred, the cells carrying Cre will cause those cells to lose the target gene.

1. Transgenic mice containing a gene surrounded by *loxP* sites are mated with transgenic mice that have the *cre* gene expressing only in one cell type.
2. The resulting mice with both the *cre* gene and the *loxP*-flanked gene.
3. In tissues with no *cre* gene the target gene with be present and function normally.
4. In a cell where Cre is expressed, the target gene of interest will be deleted.

Therefore, if the *cre* gene is bound to a promoter that only allows Cre production in neuronal cells, the target

gene will be deleted only in those cells. This method allows researchers to isolate the effects of genes in specific tissues, thereby providing very specific analysis of gene function. Since the Cre/lox system has been used extensively over the last fifteen years, there are now numerous animal, plant and bacterial stocks that already contain the *cre* gene driven by ubiquitous or tissue-specific promoters. These established lines provide a quick method for breeding experiments like those described above.

## Advantages and Disadvantages

The Cre/*lox* system has the advantage of working in almost any type of cell. This versatility has led to its application in many types of experiments, such as, labelling neuronal cells in the brain, differentiating them from surrounding glial cells and looking at the properties of promoters[5]. A more powerful approach to controlling gene expression has also been developed by combining the Cre/*lox* and the tetracycline-regulated transcriptional systems[6]. In this combination system expression of Cre occurs only when the drug tetracycline is administered, allowing researchers to start and stop expression of the gene at any time. The two primary disadvantages of the Cre/*lox* system are that not all tissue specific promoters are perfectly specific[4]. Basal levels of expression in other cell types can sometimes cause unintended gene expression. In addition, the establishment of transgenic systems with inserted genes requires a significant amount of time and money.

## A Final Thought

The Cre/*lox* system is an invaluable tool for molecular biology. By establishing tissue specific expression, it allows the isolation of individual genes and their functions. Controlling genes through the Cre/*lox* system is comparable to controlling a toy car. You can select the area where the gene will be expressed (direction) and control the level (speed) at which the gene will be expressed.

### Additional Reading


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Thesaurus

One entry found for **inflammation**.

Main Entry: **in·flam·ma·tion**
Pronunciation: `"in-fl&-'mA-sh&n`
Function: *noun*
**1 :** a local response to cellular injury that is marked by capillary dilatation, leukocytic infiltration, redness, heat, and pain and that serves as a mechanism initiating the elimination of noxious agents and of damaged tissue
**2 :** the act of inflaming : the state of being inflamed

For **More Information on "inflammation" go to Britannica.com**
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Pronunciation Symbols







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D

The American Heritage® Dictionary of the English Language: Fourth Edition. 2000.

# inflammation

SYLLABICATION: in·flam·ma·tion

PRONUNCIATION: ˌĭn'flə-mā'shən

NOUN: **1.** The act of inflaming or the state of being inflamed. **2.** A localized protective reaction of tissue to irritation, injury, or infection, characterized by pain, redness, swelling, and sometimes loss of function.



CONTENTS · INDEX · ILLUSTRATIONS · BIBLIOGRAPHIC RECORD
< inflammable inflammatory >

# inflammation

<pathology> A localised protective response elicited by injury or destruction of tissues, which serves to destroy, dilute or wall off (sequester) both the injurious agent and the injured tissue.

It is characterised in the acute form by the classical signs of pain (dolor), heat (calor), redness (rubor), swelling (tumour) and loss of function (functio laesa).

Histologically, it involves a complex series of events, including dilatation of arterioles, capillaries and venules, with increased permeability and blood flow, exudation of fluids, including plasma proteins and leucocytic migration into the inflammatory focus.

Origin: L. Inflammatio, inflammare = to set on fire

(11 Jun 1998)

**Previous**: infirmary, infirmity, infix, inflame, inflamed, inflamed ulcer, inflammable
**Next**: inflammation: gallium imaging, inflammation mediators, inflammatory